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DOCUMENT-IDENTIFIER: US 6245227 B1

TITLE: Integrated monolithic microfabricated electrospray  
and liquid  
chromatography system and method

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US-CL-CURRENT: 210/198.2, 204/600 , 210/243 , 210/656 , 210/748  
, 250/288

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PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS This application is  
related to copending U.S.

application Ser. No. 09/156,507, entitled INTEGRATED  
MONOLITHIC

MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY  
SYSTEM AND METHOD, filed

Sep. 17, 1998.

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DWKU:

**6245227**

BSPR:

Liquid chromatography (LC) is a well-established analytical  
method for

separating components of a fluid for subsequent analysis  
and/or identification.

Traditionally, liquid chromatography utilizes a separation  
column, such as a

cylindrical tube, filled with tightly packed beads, gel or  
other appropriate

particulate material to provide a large surface area. The  
large surface area

facilitates fluid interactions with the particulate  
material, and the tightly

packed, random spacing of the particulate material forces  
the liquid to travel

over a much longer effective path than the length of the  
column. In

particular, the components of the fluid interact with the  
**stationary phase** (the

particles in the liquid chromatography column) as well as the mobile phase (the liquid eluent flowing through the liquid chromatography column) based on the partition coefficients for each of the components. The partition coefficient is defined as the ratio of the time an analyte spends interacting with the stationary phase to the time spent interacting with the mobile phase. The longer an analyte interacts with the stationary phase, the higher the partition coefficient and the longer the analyte is retained on the liquid chromatography column. The components may be detected spectroscopically after elution from the liquid chromatography column by coupling the exit of the column to a post-column detector.

BSPR:

Capillary electrochromatography is a hybrid technique which utilizes the electrically driven flow characteristics of electrophoretic separation methods within capillary columns packed with a solid stationary phase typical of liquid chromatography. It couples the separation power of reversed-phase liquid chromatography with the high efficiencies of capillary electrophoresis. Higher efficiencies are obtainable for capillary electrochromatography separations over liquid chromatography because the flow profile resulting from electroosmotic flow is flat due to the reduction in frictional drag along the walls of the separation channel when compared to the parabolic flow profile resulting from pressure driven flows. Furthermore, smaller particle sizes can be used in capillary electrochromatography than in liquid chromatography because no back pressure is generated by electroosmotic flow. In contrast to electrophoresis, capillary electrochromatography is capable of separating neutral molecules due to analyte partitioning between the

stationary and mobile  
phases of the column particles using a liquid  
chromatography separation  
mechanism.

DEPR:

The plurality of separation posts 416 extends from a side wall of the separation channel 412 in a direction perpendicular to the fluid flow through the separation channel 412. Preferably, one of the ends of each separation post 416 does not extend beyond and is preferably coplanar or level with the second surface 417. The separation channel 412 is functionally similar to the liquid chromatography column in that component separation occurs in the separation channel 412 where the plurality of separation posts 416 perform the liquid chromatography function. Component separation occurs through the interaction of the fluid flowing through the separation channel 412 wherein the columnar separation posts 416 provides the large surface area. The surfaces of the separation channel 412 and the separation posts 416 are preferably provided with an insulating layer to insulate the fluid in the separation channel 412 from the substrate 402. Specifically, the separation posts 416 are preferably oxidized silicon posts which may be chemically modified using known techniques in order to optimize the interaction of the components of the sample fluid with the stationary phase, the separation posts 416. In one embodiment, the separation channel 412 extends beyond the separation posts 416 to the edge of the substrate 402 and terminating as the exit orifice 414.

DEPR:

Further, all features such as the reservoir, the separation channel and the separation posts are recessed from the substrate 402. The portion of the substrate 402 exterior to the reservoir and the separation

channel thus serves to physically protect the separation posts from casual abrasion and stress fracture in handling and subsequent bonding of the substrate 402 and the cover 420. Because the posts are integral with the substrate, the posts are inherently stable and thus allow for the use of a pressurized system without the risk of damage to the stationary phase which may otherwise result with the use of conventional packing materials in conventional high-performance liquid chromatography systems.

DEPR:

After the fluid samples pass the SPE posts 416L, waste products from, for example, the solid-phase extraction process may be directed into a waste reservoir 410Q. In particular, during the SPE process, voltage differences may be applied between or amongst reservoirs 410M, 410N, 410P, and 410Q such that a portion of the fluid from reservoirs 410M, 410N is directed to waste reservoir 410Q while the remaining portion of the fluid from reservoir 410M remain on the SPE posts 416L. Material may then be washed off of the SPE posts 416L by directing fluid from, for example, reservoir 410P through channel 412M for separation of the extracted material by separation posts 416M. Additional reservoirs 410R, 410S downstream of the waste reservoir 410Q and upstream of the separation posts 416M may be provided to contain gradient elution of analytes in one reservoir and a diluent in the other reservoir. Gradient elution facilitates chromatography by changing the mobile phase composition, i.e. the polarity to facilitate analyte interactions with the stationary phase, and thus facilitate separation of the analytes. In addition, the diluent provides the correct polarity of the solution for the next separation.

